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High-quality assemblies of five phylogenetically diverse snakes

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Abstract

Objectives:

Snakes (suborder Serpentes) represent a wide range of reptile biodiversity, comprising >4,000 species exhibiting distinctive morphological, ecological, and physiological adaptations compared with other squamates. However, comparative genomic studies in snakes remain constrained by the limited number of high-quality genomes. In this study, we aim to mitigate this issue by providing high-quality genomes for five phylogenetically diverse species.

Data description:

We obtained an average of 84.34 Gbp CycloneSEQ reads and 78.17 Gbp of DNBSEQ short reads and assembled high-quality genomes of five snake species, including *Python bivittatus*, *Trimeresurus albolabris*, *Bungarus fasciatus*, *Oligodon formosanus,* and *Coelognathus radiatus*. Completeness assessment with Merqury k-mer and Compleasm BUSCO yielded an average completeness of 96.95%

and 96.44%, respectively, with QV exceeding 40. Genome annotation using homology and *de novo* prediction resulted in an average of 22,623 genes and 683.13 Mb repetitive sequences, occupying 44.45% of the snake genomes in average.

Keywords

Serpentes, high-quality genome, genome assembly, genome annotation, long-read sequencing

Objective

Snakes constitute a major vertebrate lineage comprising more than 4,000 extant species distributed across diverse ecosystems [1]. Over ~100 million years of evolution after diverging from other squamata, snakes have undergone remarkable morphological and physiological changes, including complete limb loss, specialized venom systems, profound sensory and feeding behavior modifications [2–5]. Decoding the genetic mechanisms of these traits provide implications for human health through drug discovery and clinical toxicology [6,7].

Rapid growth of genome sequencing has greatly advanced our understanding of snake biology and evolution [1,8]. However, genomic resources remain limited compared to their extraordinary species richness. Around 180 snake genomes are available at NCBI (access date: 2026/05/05), representing only ~4.5% of known species. Less than one-third of these assemblies exceed the EBP recommendation of contig N50 > 1Mb [9–11]. The limited availability of high-quality genomes hampers our ability to resolve the complex genomic regions of snakes with large clusters of tandemly duplicated genes that underlie key evolutionary innovations in snakes [12–14].

To mitigate this issue, we generate high-quality genomes using CycloneSEQ long reads [15] for five snake species collected in Hong Kong, China, including *Python bivittatus* (Pythonidae), *Trimeresurus albolabris* (Viperidae), *Bungarus fasciatus* (Elapidae), *Oligodon formosanus* and *Coelognathus radiatus* (Colubridae). *P. bivittatus* represents an early-diverging lineage of large-bodied nonvenomous snakes [16] and provides the basal snake genomic architecture [17]. *T. albolabris* and *B. fasciatus* represent two major lineages of venomous snakes with distinct venom systems and ecological strategies [5,18]. Finally, *O. formosanus* and *C. radiatus* both represent the most species-rich family Colubridae [19] but exhibit distinct feeding and ecological adaptations [20,21]. Together, these species encompass substantial phylogenetic breadth and ecological roles, including both venomous and nonvenomous predatory strategies, microhabitat adaptations, and diverse dietary specializations, providing complementary resources for snake comparative genomics studies.

Data description

All five snake samples were collected in Hong Kong, China, euthanized with an overdose of MS-222 [22], and stored at -80 °C until DNA extraction. Genomic DNA was extracted using CTAB from liver tissues. WGS libraries were prepared according to manufacturer's instructions [15,23–26] for CycloneSEQ and

DNBSEQ. Across five species, we generated an average of 84.34 Gbp CycloneSEQ long reads (~60× genome coverage; read N50 = 7.91 kbp) and 78.17 Gbp (~55x coverage) PE DNBSEQ short reads (Table 1, Data set 1). For *O. formosanus* and *T. albolabris*, remaining liver tissues were used for Hi-C library preparation following the Hi-C 2.0 protocol, yielding an average of 57.52 Gbp (~42x coverage) data per species (Data file 1).

Draft genome assemblies were generated from CycloneSEQ reads using NextDenovo (v2.5.2) [27] and polished with DNBSEQ reads using NextPolish (1.4.1) [28]. The resulting assembly sizes ranged from 1.43 Gbp (*P. bivittatus*) to 1.58 Gbp (*O. formosanus*), and exhibited high continuity (contig N50 ranged from 7.36 Mbp in *B. fasciatus* to 57.14 Mbp in *P. bivittatus*; Data file 2). We further used YaHS (v1.1.2) [29] with Hi-C data to scaffold *O. formosanus* and *T. albolabris'* assemblies, achieving scaffold N50 of 211 Mbp and 266 Mbp, respectively. For both species, >99% of assemblies were anchored into 18 chromosomes, including 8 macro- and 10 micro-chromosomes (Data files 3 & 4), matching the typical karyotype of *Serpentes* [30–32]. We evaluated assembly completeness and accuracy using Merqury (v1.3.0) [33] and Compleasm (v2.6.0) [34]. The five assemblies showed high k-mer completeness (range=95.84-98.31%) and high BUSCO completeness scores (range=96.16-97.94%) based on the sauropsida_odb10 (Data set 2). QV scores estimated with short reads k-mer were all above 40, indicating base-pair accuracies more than 99.99%.

Repeat elements were annotated using RepeatMasker (v4.1.2) [35] and RepeatModeler (2.0.5) [36]. The total non-redundant length averaged for 683.13 Mbp and accounted for 44.45% of the genome (range=33.02-49.57%; Data file 5). LINE and DNA transposons were the most abundant repeat classes in all the genome. Protein-coding genes were annotated using EviAnn (v2.0.2) [37] and BRAKER3 (v3.0.8) [38] by integrating *ab initio* predictions with homology-based evidence from three published snakes—*Crotalus adamanteus* (GCA_039797435.1), *Candoia aspera* (GCA_035149785.1), and *Ahaetulla prasina* (GCA_028640845.1)—and *Gallus gallus* (GCF_016699485.2). We annotated an average of 22,623 genes per genome with mean gene lengths ranging from 31,387 to 35,870 bp and the CDS length ranging from 1,367 to 1,575 bp (Data file 6). BUSCO assessment of the protein-coding gene annotation with 'sauropsida_odb10' database reported a completeness score of more than 90% for all five snakes (Data set 3).

Table 1: Overview of data files and data sets.

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 1	Raw reads, assemblies, and annotation for all species	FASTQ (.fastq), FASTA (.fasta) and GFF (.gff) file	Genome Sequence Archive (GSA) (https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA069040) [39]
Data set 2	Compleasm BUSCO assessment of genome assembly	Zip folder(.zip)	Figshare (https://doi.org/10.6084/m9.figshare.32254497)[40]
Data set 3	Compleasm BUSCO assessment of protein coding gene annotation	Zip folder(.zip)	Figshare (https://doi.org/10.6084/m9.figshare.32974640)[41]

Data file 1	Statistics of CycloneSEQ, DNBSEQ and Hi-C data	Spreadsheet (.xlsx)	Figshare (https://doi.org/10.6084/m9.figshare.32253921)[42]
Data file 2	Statistics of genome assembly	Spreadsheet (.xlsx)	Figshare (https://doi.org/10.6084/m9.figshare.32254296)[43]
Data file 3	Hi-C contact map and chromosome assignment of *O. formosanus*	PDF file (.pdf)	Figshare (https://doi.org/10.6084/m9.figshare.32254731)[44]
Data file 4	Hi-C contact map and chromosome assignment of *T. albolabris*	PDF file (.pdf)	Figshare (https://doi.org/10.6084/m9.figshare.32254734)[45]
Data file 5	Statistics of repeat annotation	Spreadsheet (.xlsx)	Figshare (https://doi.org/10.6084/m9.figshare.32254341)[46]
Data file 6	Statistics of protein coding gene annotation	Spreadsheet (.xlsx)	Figshare (https://doi.org/10.6084/m9.figshare.32254371)[47]

Limitations

Although the five snake genomes achieved high contiguity, chromosome-level assemblies were generated only for two species due to insufficient materials for Hi-C. The remaining three assemblies are therefore limited to contig level, which may constrain analyses requiring chromosome-scale information, such as ancestral karyotype reconstruction and chromosomal rearrangements. In addition, sex determination systems and sex chromosome identities are highly diverse across snakes, making the clade an informative system for investigating the origin, differentiation and turnover of sex chromosomes [48]. However, the sex of sampled individuals was not recorded during collection, which hinder the identification and analysis of sex chromosomes in the present study. Nevertheless, we anticipate that the draft genome assemblies, together with the genome annotations generated in this study, will serve as valuable resources for phylogenomics and comparative genomics for future snake evolution studies.

Abbreviations

BUSCO	Benchmarking Universal Single-Copy Orthologs
CDS	Coding DNA Sequence
CTAB	Cetyltrimethylammonium bromide
EBP	Earth BioGenome Project
EviAnn	Evidence-based Annotation
Hi-C	High-throughput chromosome conformation capture
LINE	Long Interspersed Nuclear Element

MS-222	Tricaine methanesulfonate (3-aminobenzoic acid ethyl ester methanesulfonate)
NCBI	National Center for Biotechnology Information
PE	Paired-End
k-mer QV	K-mer-based Quality Value (sequence quality estimation using k-mer frequency)
WGS	Whole Genome Sequencing
YaHS	Yet another Hi-C Scaffolding tool

Declarations

Ethics approval and consent to participate

This study was conducted under the permits issued by the Government of the Hong Kong Special Administrative Region (HKSAR) [(161) in AF GR CON 09/51 Pt.7], and methods were approved by the HKSAR Government [Permit # (20-51) in DH/HT&A/8/2/8 Pt.1) and Lingnan University Ethics Committee [EC050/2021], and ethical approval provided by The Institutional Review Board of BGI, China (BGI-RO29-1-T4). All procedures followed the guidelines and regulations outlined in the approval document. Consent for sample collection and use, as well as the publication data in genomic research was obtained by BGI in accordance with the approved ethical protocol. Documentation confirming participant consent was provided by BGI as part of the ethics permit.

Consent for publication

Not applicable

Availability of data and materials

The data described in this Data note can be freely and openly accessed on the Genome Sequence Archive (GSA) in the China National Center for Bioinformatics (CNCB) under accession number PRJCA069040 (https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA069040). Please see Table 1 and references [39–47] for details and links to the data.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

K.S.L conducted formal analysis, visualizations, methodology, software, data curation, and writing the draft. W.J. performed sample investigation and methodology, A.F. pilot the investigation. J.J.F. led the conceptualization, managed resources, funding acquisition, and reviewed and edited the draft. J.J. and D.F. conducted a part of methodology. S.L., Z.Y., W.L. and C.Y. provided sequencing resources. Q.L. supervised and investigated the research. Y.Z. administered the project, software, formal analysis, draft review and edit, and funding acquisition. All authors read and approved the final submission.

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